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WATERBORNE TRANSMISSION OF GIARDIASIS

WATERBORNE TRANSMISSION OF GIARDIASIS

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Abstract

A general review of the organism Giardia sp. is presented to familiarize hydrologists with the organism, its impact upon humans, and its occurrence in the natural environment. Also discussed are current efforts to sample for Giardia sp., anticipated data needs and the potential role of the U.S. Forest Service in Giardia sp. studies.

An adaptation to the wildland setting of the existing EPA sampling technique is described along with an initial prototype evaluation. Included are figures describing the prototype Forest Service In-stream Giardia Sampler design and applications.

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I The Disease

Giardia lamblia, a flagellated protozoan, is the most commonly diagnosed flagellate of the human intestinal tract.[1] This organism, while endemic to most of the world, has become an item of increasing concern to wild-land recreationists especially to those of the Rocky Mountain area. National attention has been paid this organism since epidemic levels were observed in American travelers returning from Leningrad and Tokyo.[2,3] In addition, epidemic level occurrences in United States resort towns (Aspen, Vail, etc.) [4,5,7,8] and other municipalities (Berlin, N.H., Camas, Wash., Boulder, CO, etc.)[6,9] have drawn further attention to the organism and its associated disease.

While the largest single documented U.S. outbreak occurred in Rome, New York in 1975,[2,16] the bulk of the noteworthy "outbreaks" have occurred in Colorado. There has been identified, however, a national increase in incidences (estimated to number 70 since the 1965 Aspen, Colorado epidemic),[10] but this may not be a real trend. Some clinicians contend the trend is in diagnosis rather than incidence.

The clinical condition produced by this organism, (termed Giardiasis) is not generally life threatening. The organism, by means of a sucking disc, attaches itself in the trophozoite form to the upper part of the small intestine (duodenal-jejunal mucosa) rarely entering the gallbladder or biliary tree.[3] Some clinicians feel significant mechanical irritation to the mucosa may be consequent to this attachment;[3] however, this has not been adequately established.

Human symptomatology includes malodorous diarrhea, flatulence (intestinal gas), anorexia (loss of appetite), malaise (weakness, discomfort), weight loss, nausea, midepigastric cramps, and abdominal distention (bloating).[3] Also associated is a malabsorption syndrome in which Vitamin A[11] is not normally absorbed by the intestinal tract. This aspect of the disease is especially pronounced in juveniles and certain ethnic groups.[3,11]

The prepatent period (time between infection and diagnosis) varies to some degree but generally averages about 9 days.[12,13] Symptoms may last indefinitely[3,12] and observers have commonly reported ranges of 5 to 41 days[12] and 2-6 weeks.[13] In addition, spontaneous remission of symptoms was reported as a common phenomenon by one observer.[12]

Symptomatic individuals tend to fall into one of three classes of symptom severity, i.e., chronic, sub-acute and acute. In the acute stage the symptoms are as described previously and may also include vomiting, chills, low grade fever, and headache. These symptoms may last from a few days to a few months. The sub-acute stage is characterized by intermittent episodes of the above but generally not of the same severity. These symptoms could also last for months.[13]

The asymptomatic is the most common form of infection. The length of time during which such an infected individual may shed cysts has not yet been determined.[13]

The disease is generally successfully treated through drug therapy. Drugs employed include quinacrine (Atabrine) hydrochloride, metronidazole (Flagyl), furazolidone (Furoxone), tinidazole (Flasigyn) (not available in U.S.), acranil, chloroquine, amodiaquin hydrochloride, and camoquin.[1,2,3,11] Of the foregoing only the first three drugs are routinely used for the treatment of Giardiasis. The success rate decreases in the order of presentation above. A similar trend in severity of side effects is also associated with these drugs. Specifically quinacrine is contraindicated for individuals with psychosis or psoriasis, and all are contraindicated for pregnant individuals.[3] Special care must be exercised in any treatment for individuals suffering from ulcers or similar gastro-intestinal disorder (i.e., gall bladder).

One point in regards to treatment which should be mentioned at this juncture is the current discussion regarding the possibility of carcinogenic side effects of metronidazole (Flagyl). If such should be validated then a principal form of treatment would become inappropriate.

It has been observed that patients who have been successfully treated utilizing chemo-therapy tend to be subject to reinfection if subsequently exposed. However, untreated patients and asymptomatic patients tend to maintain a degree of immunity to reinfection.[14]

Some question remains as to the mode of transmission of the organism and the dosage required to induce clinical symptoms. It is, however, generally accepted that this organism is, at the minimum, waterborne. This was clinically documented for the first time in Rome, New York in 1975.[2,9] Early research with prisoner volunteer subjects indicated encapsulated dosages of as little as 10 organisms (cysts) could be adequate to produce clinical symptoms but left unanswered questions as to dosage and mode of natural transmission.[12]

II The Organism

Giardia lamblia was first observed in 1681 by Leeuwenhoek in a microscopic examination of his own stools. Recognizably precise description, however, was not accomplished until 1859 when Lambl gave the organism the name intestinalis. Subsequent confusion over naming was, in part, eliminated by Stiles in 1915 who created the binomial Giardia Lamblia in honor of Professor A. Giard of Paris and Doctor F. Lambl of Prague.[1] Stiles' efforts, however, have not entirely eliminated the confusion. This is primarily a consequence of imperfect knowledge of the organism's morphology.

Early researchers felt the species of Giardia were highly host specific. Consequently there arose the species names bovis (cattle), caprae (sheep), canis (dog), cati (cat), duodenalis (rabbit), caviae (guinea pig), muris and simoni (Norway rat) and muris and microti (house mouse).[15] Recent cross transmission studies and electron microscopic examination have cast substantial doubt upon this classification schema.[16] There is currently a tendency to identify only two or three species of Giardia.[13,14] However, further study is required.

Giardia Lamblia has been phylogenetically classified as follows:[1,13]

Kingdom: Animalia - Linnaeus, 1758

Phylum: Protozoa - Goldfuss, 1818, emend. Von Siebold, 1895

Subphylum: Sarcomastigophora - Honigberg and Balamuth, 1963

Superclass: Mastigophora - Diesing, 1866

Class: Zoomastigophora - Calkins, 1909

Order: Protomonadina

Family: Hexamitidae

Genus: Giardia

Species: Lamblia - Stiles 1915,

et. al.

The organism has a two stage life cycle characterized by cystic and trophic stages. The mature cyst tends to have four nuclei situated at one end of the organism and divides to form two individuals when excystation occurs. The cyst is ovoid or ellipsoid and measures 8-12 μm by 6-10 μm.

The trophozoite is pear shaped, bilaterally symetrical and measures 9-21 μm long; 5 to 15 μm wide and 2-4 μm thick. The trophozoite is also characterized by two nuclei, four pairs of flagella and a notched concavity which occupies virtually all of the anterior-ventral half of the organism.[1,13] This concavity is the sucking disc which attaches to the duodeneal-jejunal mucosa. It has been postulated that the ventral flagella serve to create a flow of liquid towards the low pressure area created by the sucking disc through a beating action which would facilitate attachment and nourishment.[27]

Reproduction in the trophozoite form is accomplished through longitudinal binary fission resulting in two daughter trophozoites. Encystation is incompletely understood at this time but is apparently triggered when

diarrhea diminishes resulting in dehydration of liquid feces during transit through the colon.

Little information is readily available on the ability of cysts to remain viable under environmental extremes. Cysts have been observed to survive in cold or tepid water for from one to three months, and at temperatures of down to -20°C for up to ten hours. The organism is, however, immediately killed at 50°C. In addition, the encysted organism is highly resistant to chlorination surviving .5 percent chlorinated water for two to three days. Iodination, on the other hand, is a more successful purification agent resulting in cyst destruction at doses normally encountered in water purification.[3,15,17]

III Giardia in the Natural Environment

As noted earlier the first instance in which the waterborne transmission of Giardia was documented occurred in Rome, N.Y. in 1975.[2,9] Consequent to the above, researchers have attributed the endemic occurrence of this organism in Colorado to the consumption of untreated mountain stream water.[18]

The lack of adequate understanding of organism morphology and classification has resulted in a degree of confusion as to the degree and avenues by which the organism moves from host to host if, in fact, such transfer occurs. This has resulted in significant cross-transmission studies by various researchers.[16,21] One of the more ambitious efforts is being carried out by Dr. Charles P. Hibler and associates at Colorado State University, Wild Animal Disease Center.

Although far from providing definitive answers encouraging results have been produced. At this time data principally indicate which animal species are infected by Giardia derived from humans. An assumption of a converse relationship is utilized as a working hypothesis but remains to be proven.[19]

Numerous animal species have been identified as hosts for Giardia, however, the first documented instance in which animal infection was clinically associated with an epidemic level occurrence of Giardiasis in humans was in Camas, Washington in the spring of 1976.[9,20] On this

occasion 3 out of 12 live-trapped animals were found to be infected with Giardia sp. All infected animals were beavers. Of interest is the following: The contaminated animals were trapped within foraging distance of the watershed providing municipal water to Camas; Giardia sp. cysts were identified in the raw water entering municipal intakes; although no human contamination was observed on the watershed itself such was observed in the area in which infected animals were trapped.[9]

In Ontario, Canada, Girardia sp. was identified in 98.8 percent of meadow voles (Microtus pennsylvanicus) and 98 percent of deer mice (Peromyscus maniculatus) livetrapped in a 1978 study.[21]

At this time initial results indicate the beaver plays a central role in the transmission of Giardia. Up to 18 percent of beavers studied have been found to be positive for Giardia sp. cysts in studies in Colorado mountain areas.[19] In addition, such beaver infection has been observed to be related to human activity. Specifically, beaver populations found to be positive for infection in areas of human activity have often been found to be negative in areas upstream of such activity.[14]

The significance of infected individuals to the spread of the organism, whether human or animal, is difficult to assess. A single human subject has been observed to excrete 2.19×10^6 cysts per gram of formed stool.[13] Consequently a few infected individuals may have substantial impact upon downstream water users.

IV Sampling for Giardia

The bulk of Giardia studies have involved stool examinations of infected individuals. These have resulted in morphological and cross-transmission studies to evaluate infectivity, species differentiation and host specificity. These studies have been additionally supported by epidemiology studies and sanitary surveys in instances of known outbreaks. Virtually all of the foregoing methodologies have been indirect in approach.

The reason for reliance upon such indirect techniques has largely been the lack of a suitable in vitro cultivation technique for organism growth from the cyst stage.[13] Lacking such a technique there is no truly suitable method by which a determination might be made as to cyst viability and infectivity, outside of experimental infection. Furthermore, cysts isolated from wild animals or environmental waters reveal little information as to potential human impact without such a technique.

Recent studies indicate the potential for near future development of in vitro cultivation techniques.[22] In addition, scanning and phased electron microscopy offer potential for surficial studies of the organism which may also aid in the evaluation of viability and infectivity of Giardia cysts.

For the time being, however, the principal method by which Giardia cysts are identified from a sample of any kind is transmission microscopic

examination of all material sampled. Viability and infectivity can only be inferred through staining techniques (e.g. eosin)[16] or experimental animal infection.

Until recently, sampling for Giardia consisted of fecal examinations only. However, to gain an understanding of the waterborne aspect of this disease the EPA and Center for Disease Control have made efforts to develop techniques to sample raw and treated water so as to isolate the organism for study.

Initial attempts to isolate Giardia in Aspen, Colorado in 1965 [23] utilized the membrane filter technique.[11,29] This technique proved unsuccessful because of the need to sample large volumes of turbid water.

Following this the CDC employed a diatomaceous earth, pressure swimming pool filter to examine water during the 1975 Rome, N.Y. outbreak.[2,9,13] This technique examined (filtered) large volumes of water (over 280,000 gallons) in 10 days.[8] The results of this study were not definitive and although large quantities of water were filtered the equipment was large and cumbersome and results were not quantitative.

In 1976 the EPA developed a filtration technique which was employed in Camas, Washington and Berlin, N.H.[13] This technique employed an orlon yarn wound filter with a porosity of 7 μm. The filter was incorporated in a plastic holder which was connected to inlet and outlet hoses and a flow meter.[13] The sampler, while in itself being compact and light weight, requires a faucet connection or pump.

In this procedure the filter is removed from the collection system after filtering an appropriate amount of water and transported to a laboratory. In the lab the orlon windings are cut from the central core and washed in distilled water. The resultant residue is then fixed with Formalin, stained with Lugol's iodine, and vacuum filtered through a series of filter screens. The residue from the final filter is washed and settled, has the supernatant removed, is centrifuged, again has the supernatant removed and finally is placed upon a light transmission microscope for examination.

Every portion of the residue must be microscopically examined by an individual well acquainted with Giardia sp. A C.D.C. report indicated 20-35 percent of public and private laboratories could not correctly identify intestinal protozoa.[13] Consequently, there is substantial room for error at this point.

In addition there is room for error throughout the sampling and processing procedure. Cysts may be missed in sampling or they may be lost in each of the washing and filtering steps. Consequently a finding of no cysts does not, in fact, mean the water is free of such organisms. Only positive samples say anything for sure and even then nothing is known regarding viability, or infectivity.

While the EPA filter technique has demonstrated advantages, the procedure is still in the developmental stages. For example, the quantity of water required for filtration was originally established at 2,000 liters (approx. 520 gal). This was based upon the following assumptions: one viable cyst is infective to humans; an individual drinks two liters of water per day; it would be desirable to detect one cyst per 20 liters (based upon research findings that a dose of 10 cysts had confirmed infectivity in humans); the efficiency of recovery of the method would be 1 percent.[13] Recent work, however, has led to recommendations changing the screen material used in processing and changing the desired sample volume to 100 gallons (i.e. due to interference in dirty water).[24]

A modification of the EPA method has been developed by David L. Rosgen of the Arapaho and Roosevelt National Forest. This technique employs the EPA filter incorporated in a small flume which is placed in the river of interest. Discharge is captured at the outlet end utilizing a graduated cylinder and a flexible hose (such as used for travel trailer holding tanks). This discharge is timed to establish a flow rate which is utilized in rating the particular installation. After a suitable time/discharge the installation is again flow rated (to establish an average discharge over the sampling interval), the sampler is removed, the sample filter is extracted and then transported on ice in a sealed bag to the laboratory for processing as described above.

Initial tests utilizing five samplers at five locations have offered promise. At four locations fecal material was collected upstream of the sampler installation. At one location the fecal material collected was from muscrat and at the remaining three the material was from beaver. The fifth location offered no fecal matter. No Giardia sp. cysts were found in any fecal matter when analyzed by the Colorado State University Wild Animal Disease Center. Analysis of the filters by the same individuals did, however, reveal cysts at two of the five locations. Of the two positive samples one was found at a location previously thought to be free of cysts as determined through beaver fecal analysis by C.S.U. researchers.

The modification of the EPA technique has certain advantages over the existing method for application to the wildland setting. Foremost of which is portability. This technique is back-packable and requires no pump or power source. In addition it tends to smooth temporal variations to provide a more representative sample (in the temporal sense). To a limited degree it can be flow proportional but only to the extent that flow is confined, the sampler receives most of the flow and there is substantial grade. Another advantage lies in the slow discharge rates associated with the sampler. The device can be installed and left without monitoring for extended periods. Consequent to this is the advantage of large sample volume.

Variations in river size or discharge can be handled through multiple samplers and/or adjusted sampling volumes.

The cost of this apparatus should not be prohibitive once out of the prototype stage (prototype cost for 10 samples was approximately $110 each in August 1978, see Figure 2).

As with any device or technique there are certain disadvantages associated with this apparatus. Specifically, long time intervals are associated with sampling (i.e., in the above mentioned pilot study flow rates ranging from .09 gal/min to .30 gal/min were observed in five samplers on moderate grade streams). Such intervals render equipment susceptible to vandalism or similar damage. In addition, such long periods without monitoring leave questions as to actual flow rates as opposed to the average values derived.

Another difficulty with this device lies in the distribution of flow through the filter. (Further modification of the prototype is underway to correct this difficulty). The principal short coming of this device, however, lies in the need for adequate grade. If grade is very small the resistance of the filter is such that flow will tend to pass around the device resulting in exceedingly low flow rates. This effect can, to some degree, be overcome by head but difficulties arise in wide, shallow, low gradient rivers.

Another difficulty with sampling wide rivers with this device is river width. When sampling such a system it is evident that a smaller sample of the population is studied than in narrow rivers (due to the small cross-sectional area of the sampler). This shortcoming can, however, be overcome by utilizing more samplers.

All-in-all the modified EPA filter sampling approach described above offers significant potential for Giardia studies in the wildland setting.

V Data Needs

The evaluation of Giardia sp. in the environment cannot be adequately completed until data is available on certain central questions. Among these are: What are the spatial and temporal distributions of the organism? Is there a seasonality or discharge dependent relationship? To what degree is man infected by Giardia passed from wild animals? To what degree are wild animals infected by man? What are "normal" baseline or endemic levels of the organism? What are the mechanisms operative in cyst transport and to what degree do they function? What time scales are involved in transport and residence? What environmental extremes render cysts nonviable or noninfectious? How many species are there and what is their host specificity?

Other questions which need additional data relate to immunity mechansisms in humans, water treatment methodologies etc. Such are beyond the scope of this presentation and shall not be addressed here.

Recent presentations by researchers and administrators indicate a significant divergence of opinion on a key issue, namely - significance. Some researchers and administrators indicate substantial concern, i.e. EPA is considering the utilization of additional or changed treatment requirements for municipal water systems or a change in indicator organisms for limitation standards (away from coliforms etc. towards other micro-biological indicator organisms).[10] Other individuals emphasize the world wide endemic nature of the organism, the lack of a life threatening hazard in the disease, a readily available treatment program, and a tendency for the disease to be self-limiting.[25]

VI The Forest Service Role in Giardia Studies

There can be a need and a place for Forest Service involvement in Giardia studies. This can, however, only be determined after an in-depth evaluation of local situations.

The most important question which must first be addressed is, "how consequential is this organism, and its associated disease, to the locale in question?" In the Colorado Rockies the answer to this question is, "very consequential" and there are seven principal reasons why: First, Colorado has been identified as the region having the highest incidence of Giardiasis in the United States. The reason why has not yet been determined.[3,26] Second, the areas experiencing epidemic level occurrences have frequently been resort areas on or adjacent to National Forests which serve influential clientel; third, most of the municipal water supplies which have been infected derive their water from watersheds within National Forest boundaries; fourth, National Forest management could feasibly have an impact upon the incidence of this disease; fifth, the National Forest recreationist is a likely target for infection; sixth, the bulk of the Colorado population resides along the Front range of the Rockies and derives its water from whatersheds on National Forest lands; seventh, a potential exists for sewage sludge application to National Forest lands.

Aside from the above one of the following questions may be the determinant in deciding the need for Forest Service involvement in Giardia studies: Is there a history of Giardiasis in the area of interest? Is the area subject to heavy recreation use which tends to seasonally tax municipal water and sewage treatment systems (e.g. ski areas)? Are there significant animal populations upstream which might serve as reservoirs for infection? Is the area of interest subjected to substantial human use with inadequate or underused sanitation facilities? Is water quality the principle land management objective?

Once it has been determined that Forest Service involvement might be desirable, the limitations of data collection should also be clearly understood. These limitations include: the errors in sampling and sample processing; the inability to say definitively that a negative sample indicates the water sampled is free from Giardia; the inability to say a positive sample indicates likelihood of infection (due to lack of knowledge about viability, infectivity and host specificity); and the inability to clearly define the source of infections (i.e. animal, human, etc.).

With the foregoing in mind there are also numerous logistical problems to be overcome. For example, a need for sample examination by qualified personnel. Potential sources of help might include: Universities; State Departments of Health; Colorado State University, Wild Animal

Disease Center; EPA and U.S.D.H.E.W. Center For Disease Control. It should be noted that initial sample preparation can be learned and accomplished by Forest Service personnel in their laboratories if certain pieces of equipment are available thus reducing cost and impact to labs doing microscopic examinations.

Sampler fabrication can be a problem but should be readily surrmounted by a local metal shop due to inherent simplicity of design (see Figure 2.).

Another and perhaps, more obvious problem involves manpower and funds. Since the modified EPA sampling procedure is very slow, at least two visits to the sampling location are required per sample. However, sampler installation is not too time consuming which results in several sampler installations per day. This must be kept in mind, however, when evaluating the trade-offs in budget allocations.

What then is there to be gained by such a study in light of all the above? The data generated can help to determine: 1) temporal and spatial distribution; 2) baseline or endemic levels; 3) point sources; 3) correlation with animal or human populations 4) impact of land management on organism abundance; and 5) the correlation of water quality parameters to organism occurrence and/or abundance.

How can these data benefit management? These data may serve an informational need (i.e. warning prospective forest users of high risk areas and preventative measures), a cooperative service (i.e., identifying high risk areas or times for downstream municipal water systems), a managerial objective (i.e. altering recreation use patterns, stream flow regimes or animal populations), a managerial service (i.e. providing water and/or sewage treatment or improving same), a land use planning/controlling strategy (i.e. controlling housing development, locating sewage disposal facilities, sewage treatment effectiveness evaluation etc.).

Furthermore, once the technical questions regarding viability, infectivity, host specificity, etc. have been adequately answered, it should be a relatively simple matter to correlate with previously acquired data to handle the more difficult and, perhaps, more fundamental questions germane to Forest management.

VII Conclusion

Giardia sp. is an organism whose impact, as those who have experienced it can attest, can be debilitating and disconcerting. Although it is endemic throughout the world it is either becoming more prevalent in the United States or more readily diagnosed. This has resulted in increased awareness and concern especially in Colorado and other states noted for their pure water and pristine environment. As a consequence, there exists both an opportunity and a need, in certain situations, for wildland hydrologists to evaluate water quality from a microbiological perspective with a view to providing assistance to both management and downstream water users.

Concomitant to this need is an imperative evaluation of the question of significance, a question which is difficult, at best, when even the experts in the field disagree upon an appropriate answer.

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APPENDIX

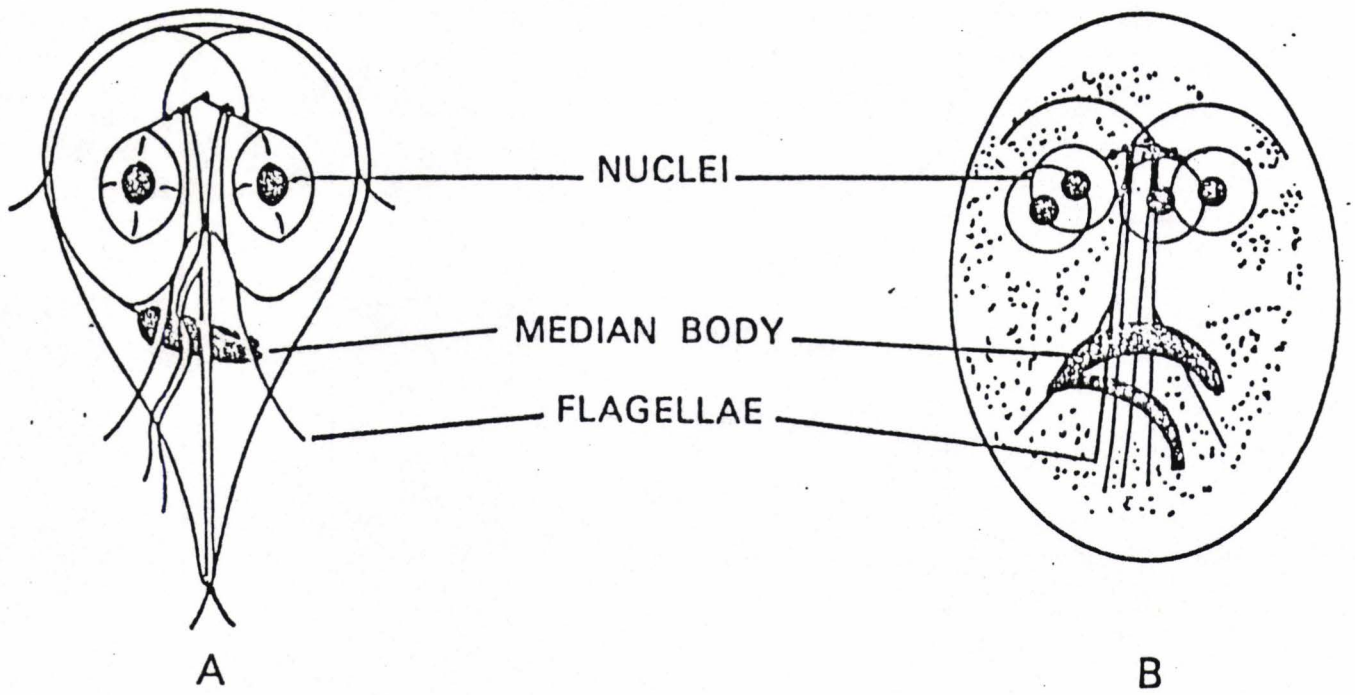
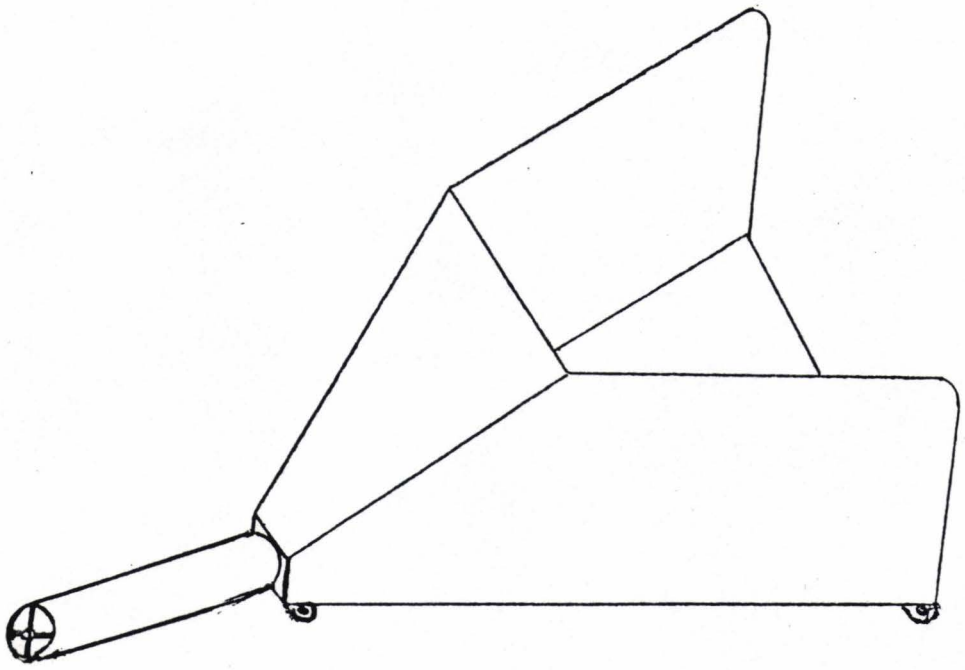


FIGURE 1. A. Giardia *trophozoite*. B. Giardia *cyst*.
(Not drawn to scale; adapted from Levine[15]*)*

Forest Service In-Stream Giardia Sampler



Not Drawn to Scale

FIGURE 2

Designed By - David L. Rosgen
Constructed By -
Oil Field Fabricators
P.O. Box 979
Fort Collins, Colo. 80522

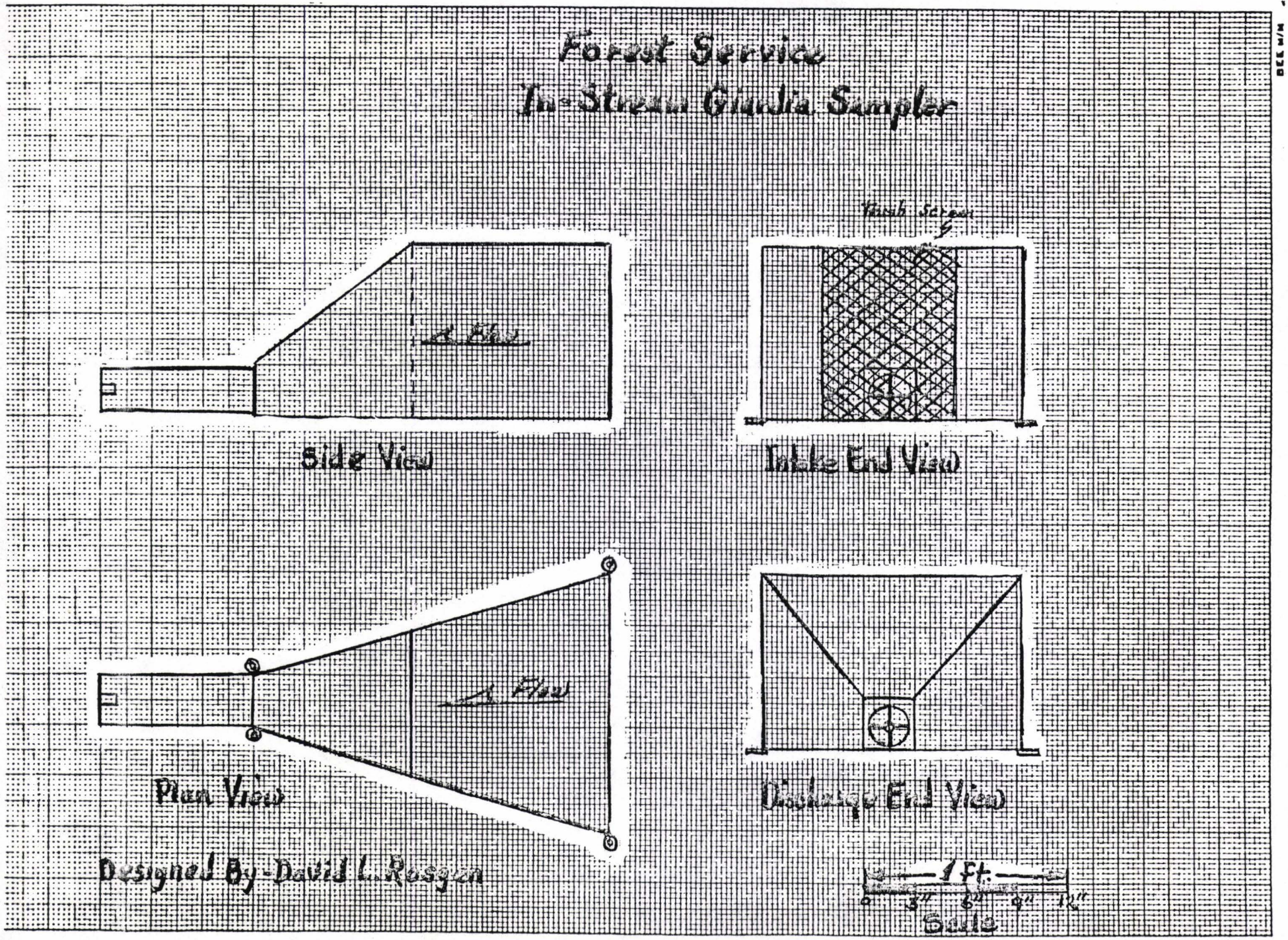
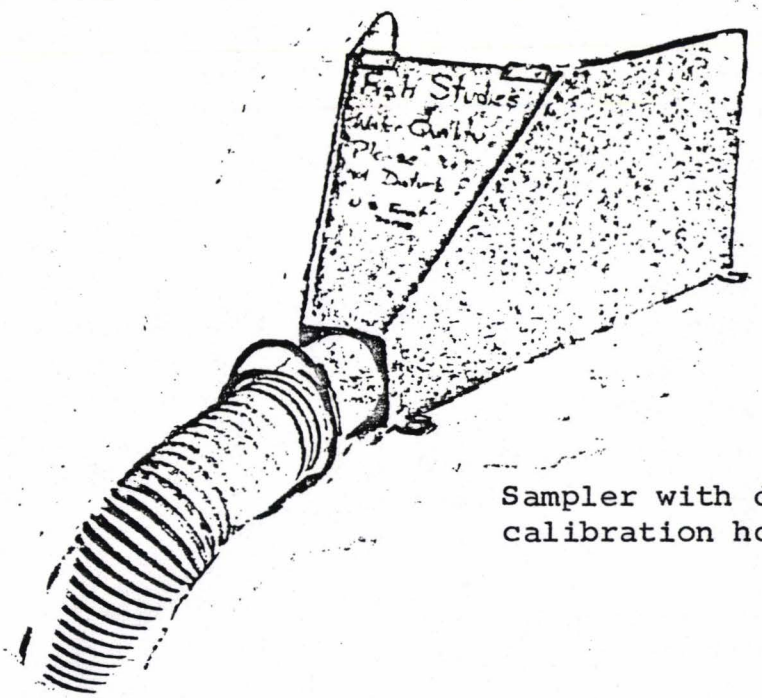

Forest Service
In-Stream Gradia Sampler
Mesh Screen
Side View
Inlet End View
Plan View
Discharge End View
Designed By - David L. Rosgen
1 Ft
0
3"
6"
9"
12"
Scale

FIGURE 3

FOREST SERVICE INSTREAM GIARDIA SAMPLER



Sampler with discharge calibration hose in place

Intake end of the sampler with trash screen lowered to working position

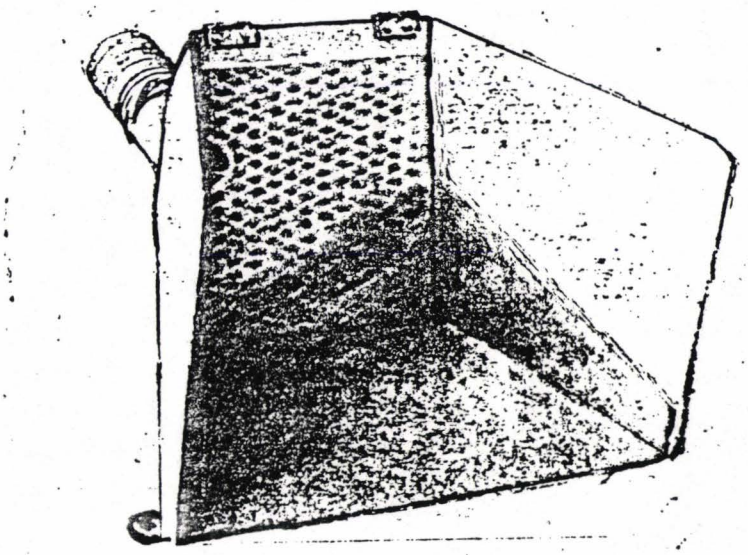


FIGURE 4

FOREST SERVICE INSTREAM GIARDIA SAMPLER - IN OPERATION

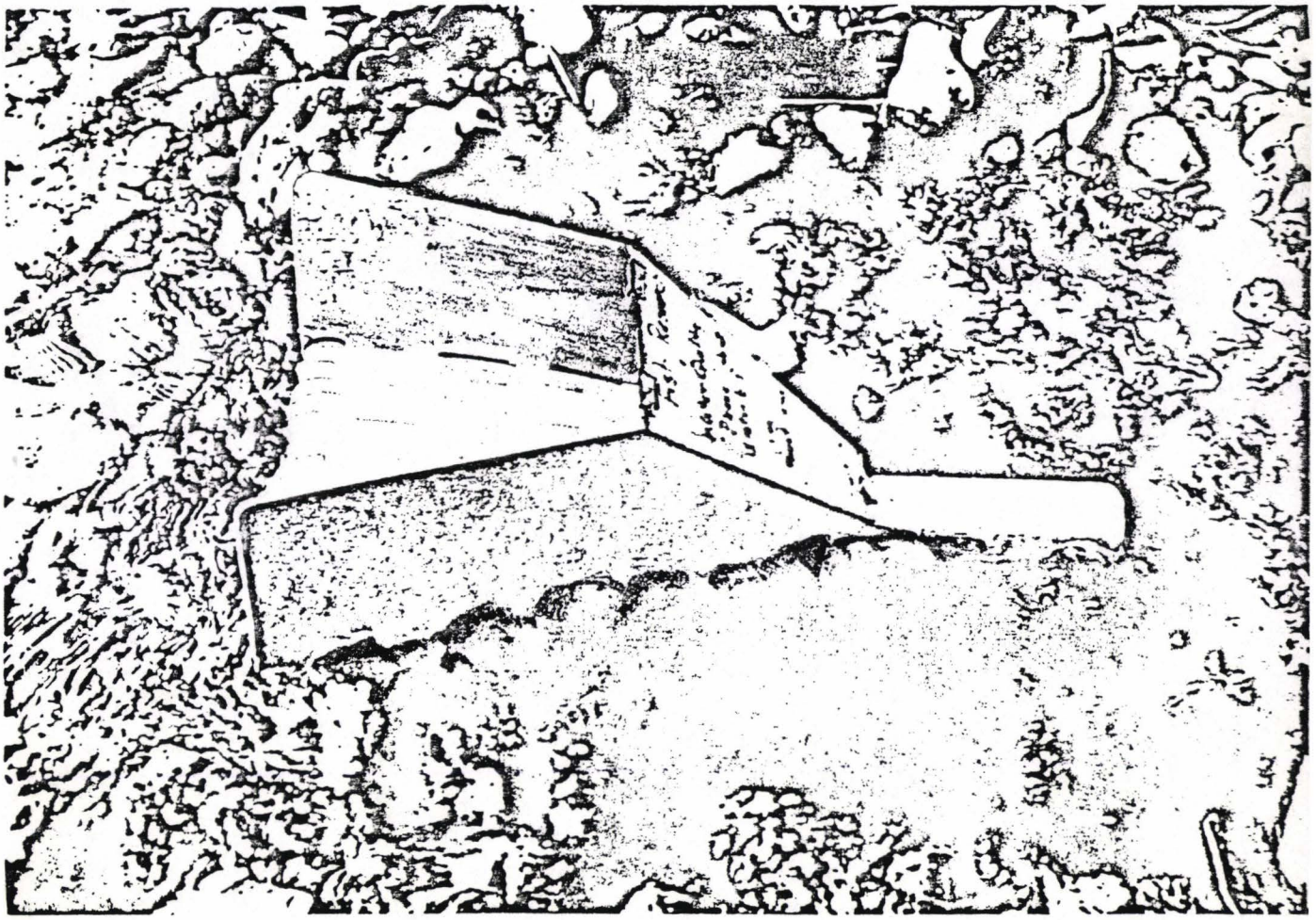


FIGURE 5